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Short Communication

Nutrient and Antinutrient Contents of Fermented Roselle Calyx

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Abstract. Pure strains of *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces cereviciae* and *Bacillus subtilis* were isolated, cultured and subsequently used to ferment four portions of 100 g each of roselle calyx for 72 h. A decrease in pH with an increase in total titratable acidity (TTA) was recorded. The results of the proximate analysis revealed a significant increase in the protein content of the fermented calyx samples (10.8 ± 1.1 - 12.6 ± 1.1) compared with the unfermented (4.8 ± 1.3). There was no significant increase in the fat content while there was a significant decrease in the ash content. There was a significant decrease ($P < 0.05$) in the antinutrient content (phytate from 2143.6 ± 0.8 mg/100 g in the unfermented sample to 488.8 ± 3.7 mg/100 g and tannin from 5.30 ± 1.1% in the unfermented sample to 1.32 ± 0.1%) after 72 h of fermentation.

Keywords: vegetable, nutrients, antinutrients, fermentation, phytochemical, biomass, *Hibiscus subdariffa*

The aim of this study is to determine the effect of fermentation on the nutrient and antinutrient content of roselle calyx fermented using cheap and non-pathogenic pure strains isolated from the traditional fermentation.

A 400 g of dry green roselle (*Hibiscus sabdariffa*) calyx was collected from a farmer at Owena near Akure. The chemicals used were of analytical grade.

Sample preparation. The roselle calyces were soaked in distilled water with 5% sodium metabisulphite added to prevent microbial growth for 6 h after which they were washed and divided into four portions of 100 g each. Each portion was put into separate bowls and 2 litres water added before in oculating with pure strains of *Aspergillus niger*, *A. flavus*, *Saccharomyces cerevisiae* and *Bacillus subtilis* (isolated, cultured and characterized from the traditional fermentation of roselle calyx by A.O. Ojokoh of the Department of Microbiology, Federal University of Technology, Akure, Nigeria). They were allowed to ferment at room temperature (27 °C) for three days.

Physiochemical changes. The pH of each sample was recorded every 12 h with a Cambridge direct reading pH meter.

Total titratable acidity (TTA) was determined on 5 ml aliquot of the sample against 0.1 M NaOH, using phenolphthalein as indicator.

Samples analysis. The proximate composition (ash, fat, moisture and crude fibre) of the fermented calyx were evaluated using the standard AOAC (1984) method and the protein content was determined using the micro-kjeldhal method (N × 6.25). The phytate content was determined using the method of Young and Greaves (1940). The tannin content was determined by the method of Makkar *et al*. (1993).

Analysis of data. The data were analyzed by students t-test (Zar, 1984). Fig. 1 and 2 show the physiochemical changes that occurred during the fermentation of the roselle calyx. The pH decrease with increase in fermentation period while the total titratable acidity (TTA) increased during the period. The decrease in pH and increase in TTA could possibly be attributed to the secretion of some organic acids during the course of fermentation by the inoculated organisms (Collard and Levi, 1959).

The result of the proximate composition is shown in Table 1. All the fermented samples had increase in protein content with *Aspergillus niger* fermented sample having the highest increase (12.6 ± 1.1).

The increase in protein content of the calyx samples could be attributed to the possible secretion of some extracellular enzymes into the calyx, in an attempt to make the use of calyx samples as a source of carbon. Apart from this, the increase in the amount of the microbial biomass in the form of single cell proteins may possibly account for the possible increase in the protein content of the samples (Ojokoh *et al.*, 2002).

The decrease in ash content in the fermented samples may be due to the utilization of minerals by the fermenting microorganisms. Table 2 shows the level of antinutrient (tannin and phytate) which the plants probably use for defense. Tannins

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Table 1. Proximate composition of calyx samples (%) fermented with indigenous isolates (mean ± SD*)

Sample	Moisture	Ash	Crude fibre	Protein	Fat	Carbohydrate
Aspergillus niger	$12.5^{c} \pm 3.1$	$5.0^{c} \pm 0.2$	$10.1^{b} \pm 0.1$	$12.6^{a} \pm 1.1$	$5.3^{a} \pm 1.0$	$54.4^{a} \pm 2.5$
Aspergillus flavus	$12.5^{c} \pm 3.5$	$4.9^{c} \pm 0.1$	$9.5^{b} \pm 0.3$	$11.8^{a} \pm 1.0$	$5.8^{a} \pm 1.5$	$55.3^{a} \pm 3.1$
Saccharomyces cerevisiae	$12.8^{c} \pm 3.5$	$5.0^{ab} \pm 0.2$	$9.0^{bc} \pm 0.6$	$10.8^{b} \pm 1.1$	$5.7^{a} \pm 1.7$	$56.6^{a} \pm 0.6$
Bacillus subtilis	$12.0^{ac} \pm 3.9$	$6.8^{ac} \pm 0.5$	$8.9^{bc} \pm 1.3$	$11.7^{a} \pm 1.6$	$3.6^{a} \pm 0.1$	$57.1^{a} \pm 1.7$
Unfermented	$11.1^{ab} \pm 1.2$	12.8 ± 2.7	$11.3^{ab} \pm 0.7$	4.8 ± 1.3	$3.9^{b} \pm 1.1$	$55.9^{a} \pm 2.3$

* = values represent means of triplicate determinations

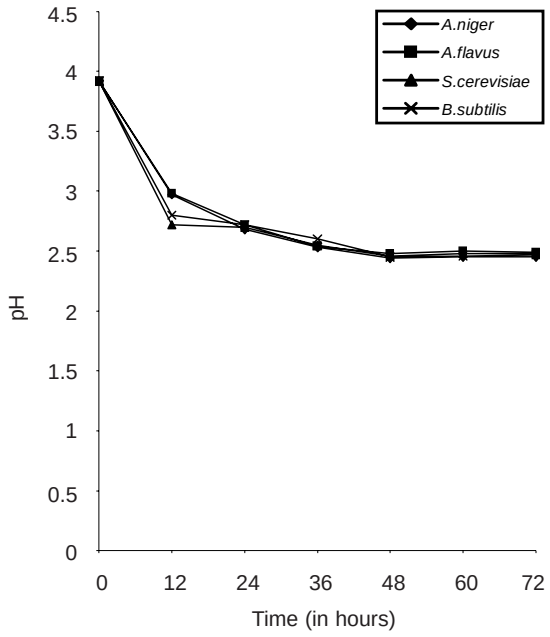

Fig. 1. Changes in pH during fermentation of roselle calyx.

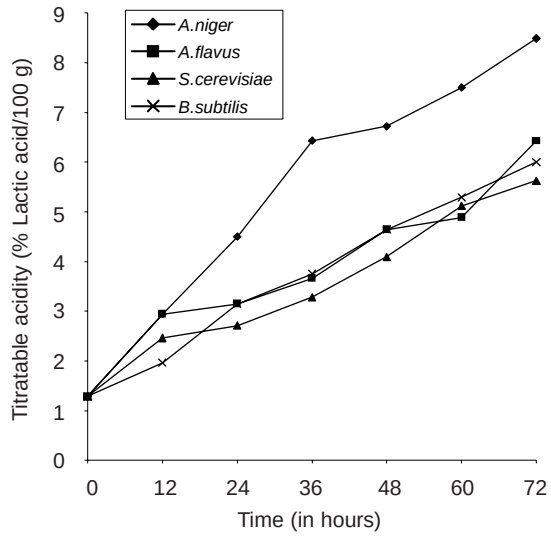

Fig. 2. Changes in titratable acidity during fermentation of roselle calyx.

Table 2. Antinutrient contents of calyx samples fermented with indigenous isolates after 72 h (mean ± SD*)

Sample	Phytate (mg/100 g)	Tannin (%)
Aspergillus niger	488.8 ± 3.7^{a}	$1.92^{ae} \pm 0.0$
Aspergillus flavus	752.4 ± 7.5^{bc}	$1.32^{bcd} \pm 0.1$
Saccharomyces cerevisiae	564.1 ± 1.1^{ab}	$1.79^{ae} \pm 0.0$
Bacillus subtilis	526.4 ± 7.5^{a}	$1.60^{ce} \pm 0.0$
Unfermented	2143.6 ± 0.8^{d}	5.30 ± 1.1^{a}

* = values represent means of triplicate determinations

affect nutritive value of food products by forming a complex with protein (both substrate and enzyme), thereby inhibiting digestion and absorption. (Aletor, 1993; Hahn, 1992). The tannin contents of the fermented calyx samples (1.32 ± 0.1 - 1.92 ± 0.0) were considerably low when compared to the unfermented sample (5.30 ± 1.1). The samples could be considered not safe in terms of tannin poisoning in view of the fact that the level of tannin is above the deleterious level (0.76 - 0.90%) as reported by Aletor (1993).

The complexing of phytic acid with nutritionally essential minerals and the possibility of interference with proteolytic digestion have been suggested, as responsible for the anti-nutritional activity (Nelson *et al.*, 1986). The phytate content of fermented calyx samples (488.8 ± 3.7 - 752.4 ± 7.5) were significantly lower than that of the unfermented sample (2143.6 ± 0.8). *Aspergillus niger* fermented sample had the highest decrease in phytate content (488.8 ± 3.7).

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